STUDIES ON INDOLE ALKALOIDS

by

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Contents.

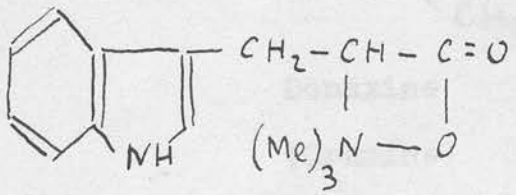
INTRODUCTION.

Among different types of alkaloids the structure of those derived from indole is least known. The constitution of only very few members of this group is fixed, and in most cases it remains obscure.

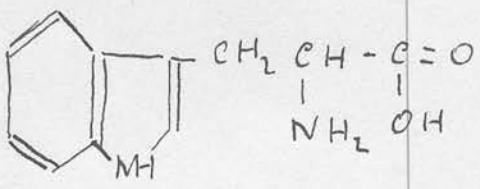
Indole alkaloids have been isolated from plants of most of the different orders of the phanerogames. Sometimes the same alkaloid is found in plants belonging to orders that have no botanical relation with each other. For instance harmane is found in Arariba rubra (N.O. Rubiaceae), Symplocos racemosa (N.O. Styraceae); yohimbine is found in Corymanthe yohimbe (N.O. Rubiaceae) and in Aspidosperma quebracho (N.O. Apocyanaceae) and calycanthine in different species of the Calycanthacae and in the Composite Maeratia praecox.

From the comparison of the structural formulae of the known alkaloids of this group it seems likely that they have originated in the plant from the amino acid tryptophane, whose structure is conserved almost intact in hypaphorine (Barger, J.C.S., 1911, 99,2068) (from Erythrina hypaphorus) and in abrine (Narendranath Ghatak/

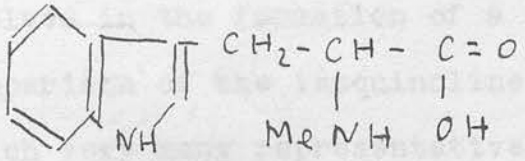
Ghatak, C., 1935, I, 576; Toshio Hashino, C., 1935, II, 3508) (from Abrus praecatorius).



Hypaporine

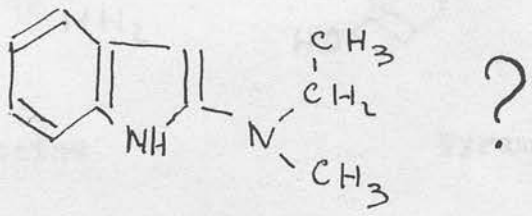


Tryptophane



Abrine

More difficult to see is the connection with the original amino acid in the suggested constitution for donaxin (von Euler, Erdmann and Hellström, Ber., 1936, 69, 743) from Arundo donax (Orechof and Norkina, Ber., 1936, 69, 436).



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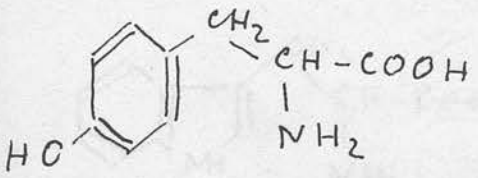
Donaxine

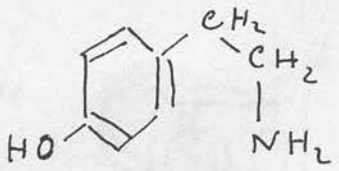
(Gramine)

In the first two cases the side chain of the tryptophane is free, but in order to explain the derivation of some other more complicated alkaloids it is necessary to assume that it has been decarboxylated and involved in the formation of a new ring.

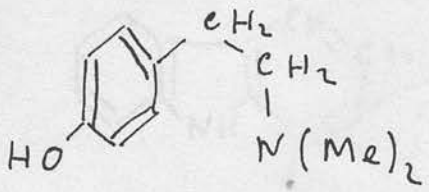
The comparison of the isoquinoline alkaloids, a group in which very many representatives are known and which are probably derived from the amino acid tyrosine, has shown that the formation of the simpler occurs by the decarboxylation of the fundamental amino acid followed in some cases by a ring closure, new atoms of carbon being involved and methylations and oxidations occurring. The closure of this ring probably takes place by reaction of the amine formed by decarboxylation of the tyrosine and an aldehyde which provides the new carbon atoms.

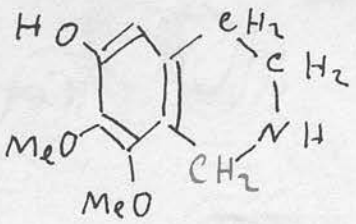


Tyrosine

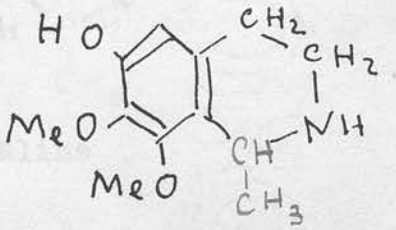


Tyramine





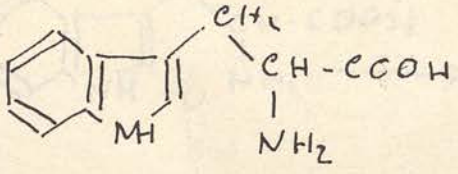
Anhalonine



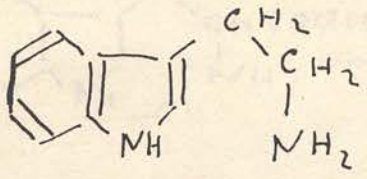
Anhalonidine

The alkaloids that illustrate the previous example have been isolated besides many others from the Cactaceae Anhalonium Lewinii. The carbon atoms marked in red are the ones from the aldehyde taking part in the condensation, namely formaldehyde in anhalonine and acetaldehyde in anhalonidine.

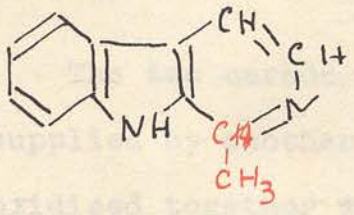
In the indole alkaloids the formation of the harmane derivatives can be explained ina similar way. The amine in this case is tryptamine and the aldehyde acetaldehyde.



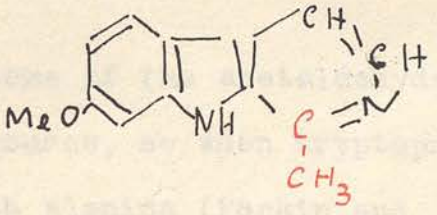
Tryptophane



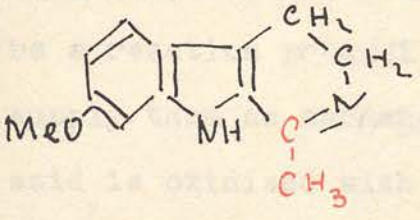
Tryptamine



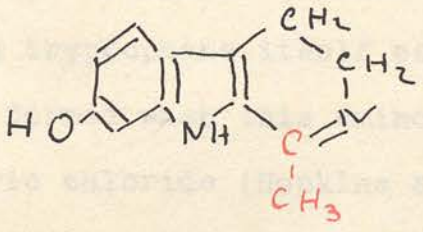
Harmane



Harmine



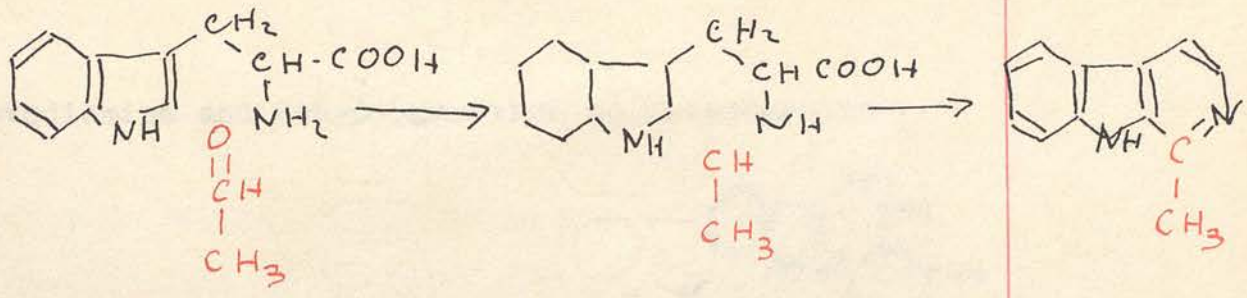
Harmaline



Harmol

In harmane and harmine complete dehydrogenation has taken place and in harmaline and harmol only partial.

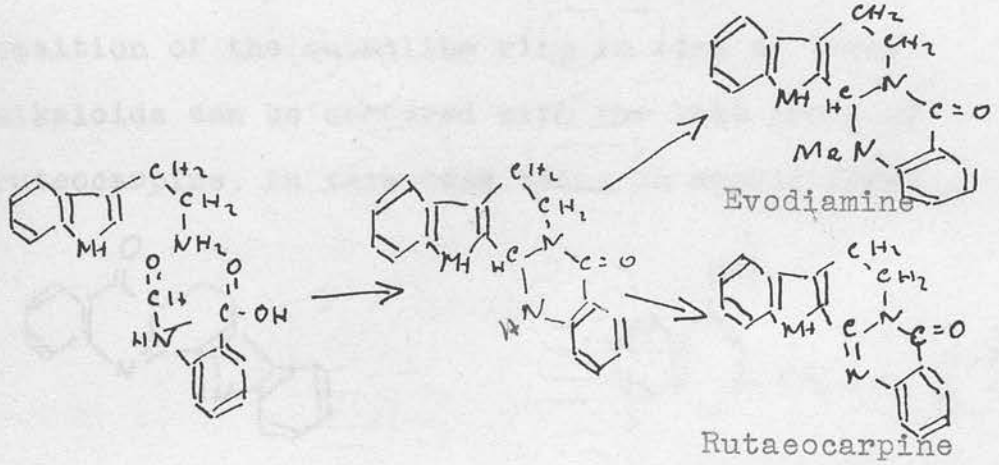
The synthesis of harmane starting with tryptophane and acetaldehyde confirms this hypothesis. By condensation of the two substances a new heterocycle is formed and by oxidation with chromic acid decarboxylated and dehydrogenated (Kermack, Perkin and Robinson, J., 1921, 119, 970).



The two carbon atoms of the acetaldehyde can be supplied by another source, so when tryptophane is oxidised together with alanine (Perkin and Robinson, J., 1921, 119, 1616) harmane is found to be a reaction product, and tryptophane itself can supply them as harmane is formed when this amino acid is oxidised with ferric chloride (Hopkins and Cole, J. Physiol. 1903, 29, 451; Robinson, J., 1919, 115, 968). This explains the formation of the very mysterious substance of Hopkins and Cole.

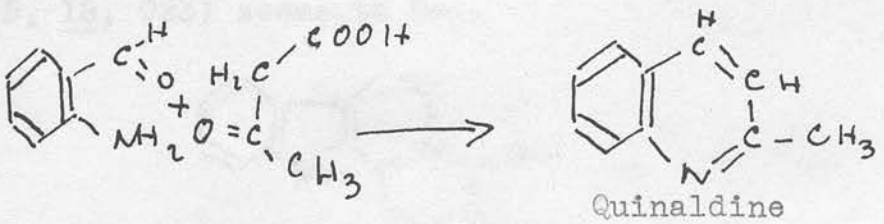
The constitution of the alkaloids of Evodia ruteacarpa (Asahina and Mayeda, Chem. Abst., 1921, I, 48), evodiamine and ruteocarpine, (the constitution of the latter only being confirmed by synthesis (Asahina, Manske and Robinson, J., 1927, 1708)), can be related to the harmane structure assuming that the condensation of tryptamine takes place with formyl anthranilic acid (or some compound related to it). A second ring closure takes place involving the carboxyl group of this acid. Methylation of the so formed hypothetical compound would yield to evodiamine/

evodiamine and dehydrogenation to ruteocarpine.

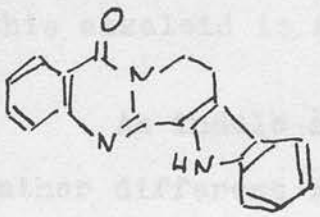


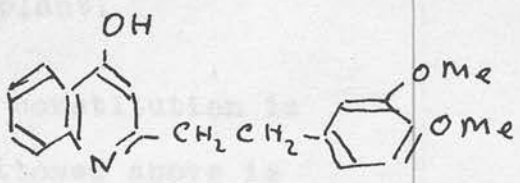
The anthranilic acid (or some related compound) can be imagined to be formed by the partial oxidation of a tryptophane molecule. The presence of methyl anthranilate in some of the essential oils proves that anthranilic acid derivatives are not foreign to the plants.

Other alkaloids that may be derived from such an oxidised indole nucleus are the quinoline alkaloids from the angostura bark. The synthesis "under physiological conditions" of some of them (Schöpf and Lehmann, Ann., 1932, 497, 7) speaks in favour of this hypothesis.



The free or methylated hydroxyl in the Y position of the quinoline ring in some of these alkaloids can be compared with the keto group of ruteocarpine, in this case being in enolic form.

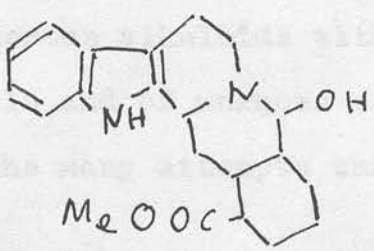




Rutaeocarpine Galipine

Other alkaloids in which the structure of the harmane is present are those from the yohimbine group, the only difference between them being the stereoisomeric arrangements in their molecules.

The formula of yohimbine has been very difficult to establish, but according to the study of the dehydrogenation products (Wibaut and Mendlik, Rec. Trav. Chim. Pays-Bas, 1931, 50, 1901; Barger and Scholz, Helv. 1933, 16, 1343; Scholz, Helv., 1935, 18, 923) seems to be:-

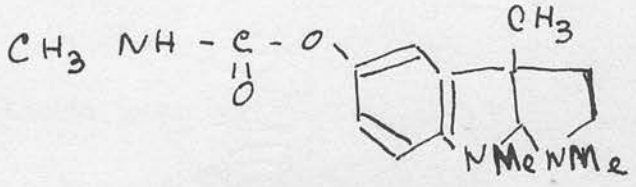


The-/

The position of the hydroxyl group is not known and the whole formula needs confirmation by synthesis.

Very little is known about the way in which this alkaloid is formed in the plant.

An indole alkaloid whose constitution is rather different from those mentioned above is physostigmine isolated from Physostigma venenosum. The formula of the alkaloid is:-



and has been confirmed by synthesis.

It is rather difficult to connect this structure with tryptophane. It is necessary to admit the methylation of the carbon atom 3; in the same way it is necessary to admit a C-methylation in order to explain the presence of the characteristic methyl group of corydaline, an isoquinoline alkaloid.

Strychnine and brucine must still be placed among the numerous alkaloids with an indole nucleus in the molecule and of unknown or doubtful constitution in spite of the many attempts that have been made

to/

to degrade them. Corynantheine, related to the yohimbine group, echitamine, quebrachamine, aspidospermine and calycanthine are further examples. The author has attempted unsuccessfully to determine the constitution of calycanthine and aspidospermine in this thesis.

ON THE ALKALOIDS OF CALYCANTHUS FLORIDUS L.

Calycanthus floridus L. belongs to the family of the Calycanthacae, a family with only a very few species occurring mostly in the form of bushes in North America, Japan and Australia. The plants belonging to this family have usually in their leaves glands with essential oils, whose principal constituents are cineol, borneol, linalool (partly esterified with formic and acetic acids), sesquiterpene alcohols, camphor, pinene and methyl salicylate.

Besides essential oils, plants of this species give off a certain amount of hydrocyanic acid which suggests the presence of a cyanogenetic glucoside, and some have a definite smell of trimethylamine. Some of the species, Calycanthus floridus and Calycanthus glaucus, have been used in medicine, their barks being employed as antipyretics (Wehmer, Die Pflanzenstoffe, 1929, p. 339).

Eccles (Proc. Amer. Pharm. Soc., 1888, 84,382) was the first to find an alkaloid in a plant of this species. He was able to isolate from Calycanthus glaucus an alkaloid that he named calycanthine. Later/

Later Gordin (J. Amer. Chem. Soc., 1905, 144) observed that cattle eating the seeds of this plant usually died. He isolated and analysed an alkaloid, probably identical with Eccles', that he thought to have the formula $C_{11}H_{14}N_2$ and he prepared a number of salts, the nitroso derivatives and studied the physiological action. The method of purification of the crude alkaloid he used is different from the one used in this work. The extraction was done in the same way by removing first the fat of the seeds by means of petroleum ether, and then extracting them with alcohol. For the purification of the alkaloid he made use of the fact that its sulphate is extremely insoluble in acetone.

The alkaloid used in this work was obtained from seeds of Calycanthus floridus L. collected in the U.S.A. They were first extracted with petroleum ether, then the alkaloidal constituents extracted by percolation with alcohol, and finally percolation with alcohol containing about 1% acetic acid until no more precipitation was given with Mayer's reagent. These operations were carried out by Messrs Duncan, Flockhart and Co. of Edinburgh, to whom I wish to express my thanks.

The/

The concentrated alcoholic extract still mixed with a little petroleum ether was extracted with ether after slight acidification and dilution in order to remove neutral and acid impurities, and then made alkaline. The great tendency of calycanthine to crystallise resulted in the alkaloids present separating almost at once as a crystalline filterable mass mixed with an oil. The solid part was fractionally crystallised from acetone and alcohol, the less soluble fractions being calycanthine; and from the mother liquors another crystalline alkaloid was isolated. This calycanthine shows identical properties with the calycanthine of Gordin (loc. cit.) and with the alkaloid isolated by Manske (J. Amer. Chem. Soc., 1929, 51 , 1836) from the Composite Meratia praecox (Rehd and Wils), which he had already identified as calycanthine. This is one of the few cases where such a complicated alkaloid has been found in species of two such different families.

The formula of calycanthine which Gordin thought to be $C_{11}H_{14}N_4$ has to be doubled after the molecular weight estimations of Späth and Stroh (Ber., 1925, 58, 2131) to become $C_{22}H_{28}N_4$ or perhaps $C_{22}H_{26}N_4$.

Barger/

Barger (private communication) had already found the double molecular weight in 1919.

In order to find out which of these formulae is the more correct, a great number of analyses has been carried out by different analysts. Nitrogen estimations were always within the limits of error; so were the hydrogen determinations, but the carbon values were always far too low, sometimes as much as 2.5% under the expected figure. In view of this fact a larger amount of the alkaloid was sent to Dr H. Roth (Heidelberg) who made a number of estimations of carbon and hydrogen. Although every precaution was taken it was always found that the figures did not add up to 100. This is due to the carbon figure. The nitrogen percentage was determined both according to Dumas and to Kjeldahl, which two methods agreed closely, and the hydrogen value was always within the limits of error. The existence of an oxygen atom cannot be accounted for. The difference between the sum of the found C, H and N figures and 100 is too small to allow its presence in a formula that would fit with the molecular weight estimations carried out by Späth.

In a private communication Dr H. Roth mentions that/

that this substance is the first he could not analyse by the micro method of Pregl. I am greatly indebted to him for the trouble he has taken in connection with the formula of this alkaloid.

In spite of all these difficulties it seems likely that the calycanthine formula is $C_{22}H_{26}N_4$.

From the estimation of the N-methyl groups it has been found that the alkaloid contains one NCH_3 which is a confirmation for the C_{22} formula suggested by the molecular weight estimation, a fact that could not be proved by analyses of the salts of the alkaloid, as calycanthine is a di-acid base.

The estimation of active hydrogen shows a rather peculiar fact. It had been done by Späth (loc. cit.) who found calycanthine to have two labile hydrogen atoms. That was confirmed in analyses made by Dr H. Roth who also found that on heating the Grignard reagent in presence of calycanthine in pyridine solution the amount of methane formed fits accurately for the calculated, assuming in the molecule the existence of four active hydrogen atoms. This is not an isolated fact in the chemistry of indole alkaloids; aspidospermin (unpublished observation) which in the cold does not react with the/

the Grignard reagent, reacts at 95° forming the exact amount of methane corresponding to one active hydrogen atom.

That calycanthine is an indole alkaloid can be easily concluded from its colour reactions. A deep blue colour appears when heated in acid solution with p-dimethylaminobenzaldehyde (Ehrlich's reagent) and it gives a very strong Adamkiewicz-Hopkins reaction (glyoxylic reagent) which suggests, with all the reserve that should be taken in the case of colour reactions that the calycanthine molecule has a free 2-position in the indole nucleus.

Besides these two colour reactions, when heated above its m.p. vapours with a strong indole-like smell are formed.

The degradation of this alkaloid done by Manske (Canad. J. Res., 1931, 4 , 275) who was able to isolate benzoyl-N-methyl-tryptamine from the oxidation product with potassium permanganate of benzoylated calycanthine, suggests that a chain formed by two carbon atoms and one nitrogen bearing a methyl are attached to the 3-position of one indole nucleus and that in the alkaloid the nitrogen atom of this particular nucleus is free as it was benzoylated/

benzoylated before the oxidation.

About the rest of the molecule very little is known. From C-methyl estimations carried out by the Kuhn-Roth method it can be concluded that this grouping is not present in the molecule.

It was impossible to show the presence of double bonds either by hydrogenation or by ozonisation. When either reaction was attempted the unchanged starting material was recovered. Not even by very long ozonisation was it possible to break the double bond 2-3 of the indole nucleus, as Scholz (loc. cit.) did in the tetrahydroyobyrine.

This makes the study of the alkaloid rather complicated as the double bond is generally a weak point against oxidising agents. The lack of such a point of attack results in most of the attempted oxidations yielding either complete destruction of the alkaloid (only oxalic acid being isolated) or in the substance being recovered unchanged, since it is rather stable against some oxidising agents.

Owing to this fact an attempt was made to dehydrogenate the alkaloid molecule in order to introduce in it either a double bond, or that one of the completely hydrogenated rings which are certainly/

certainly present in the molecule becomes a fully dehydrogenated one, the latter having more chance of surviving an oxidation.

On attempting to dehydrogenate calycanthine with mercuric acetate according to the method of Gadamer (Arch. Pharm., 1915, 253 , 274) it was found that the calycanthine was always recovered unchanged although entirely different conditions were used.

Silver acetate was used instead of mercuric acetate as suggested by Tafel (Ber., 1892, 25, 1620) and recently used by Orechoff (Ber., 1931, 64, 273) and in the dehydrogenation of anabasine, but all the experiments in this direction failed; in all cases the unchanged alkaloid was recovered.

Dehydrogenation with silver sulphate was also attempted as Tafel (Ber., 1892, 27, 826) was able to dehydrogenate some hydrogenated indoles such as methyl-ketole and dihydro-skatole that could not be dehydrogenated with mercuric or silver acetate, but in the case of calycanthine the unchanged alkaloid was recovered.

As Akabori and Saito (Ber., 1930, 63, 2246) could dehydrogenate tetrahydro-harmane and tetrahydro-harmine by means of palladium black and maleic acid/

acid and Majima and Murahashi (Chem. Abst. 1934, 1116) dehydrogenated yohimbine with palladium black and maleic anhydride, attempts were made to dehydrogenate calycanthine with these reagents, but all the experiments failed, the starting material being always recovered.

As some substances such as piperidine, tetrahydro-isoquinoline, which could not be dehydrogenated by maleic acid and palladium but were easily dehydrogenated with palladium and safrol, anethol and eugenol (Arkabori and Saito, C., 1929, II, 2033) it was tried to dehydrogenate calycanthine by refluxing with eugenol and palladium black, but the unchanged alkaloid was again recovered.

The dehydrogenation with lead tetra-acetate, being used successfully by Hahn (Ber., 1934, 67, 686) to dehydrogenate yohimbine, failed also. If a small amount was used the starting material was recovered unchanged, and if larger amounts were employed and heated for a long time no definite crystalline products were obtained.

Dehydrogenation at high temperature with zinc dust, selenium, palladium black, and lead oxide, gave in very bad yield a crystalline substance which was also obtained by dry distillation of calycanthine. It/

It is thus possible that these substances supposed to act as dehydrogenating agents only acted by diluting the alkaloid and thus allowing it to be heated regularly. During these operations the resistance of calycanthine to high temperatures could be noticed. Heated at normal pressure just over the m.p. it sublimes and is decomposed to a relatively small extent to indole-like smelling substances.

The compound obtained by these reactions has a formula of $C_{16}H_{10}N_2$ and shows weak basic properties; it is soluble in dilute hydrochloric acid with yellow colour, but forms no hydrochloride. It was never possible to observe any fluorescence of this compound in organic solvents (in most of which it is very little soluble) or in the solutions in mineral acids, not even under the ultra-violet lamp. The substance has m.p. 297° and sublimes at normal pressure far below it without the slightest decomposition. All the reactions in which this substance was formed gave a very poor yield (3-4%). There was only a very little material available so that only analyses could be made. The N-methyl group present in calycanthine has been lost and the active hydrogen estimation showed one labile atom.

In/

In order to explain the difficulties in the carbon estimation, calycanthine was mixed with copper oxide and heated together. Amongst the substances which distilled this substance of m.p. 297° was in a relatively large amount. Its formation in this reaction may be an explanation of the fact that the carbon values in the analyses were always too low, as this substance is very rich in carbon and being extremely volatile could easily have escaped unchanged in the combustion.

The dehydrogenation with selenium that gives such good results when applied to yohimbine (Wibaut and Mendlik, loc. cit.; Barger and Scholz, loc. cit.) failed completely with calycanthine. If the same conditions as in yohimbine or if higher temperature or longer heating were used, no definite crystalline substance could be obtained from the reaction product. Only by distilling the alkaloid mixed with selenium the same substance as in the zinc dust distillation could be isolated in extremely small quantities from the distillate.

As all the attempts to dehydrogenate calycanthine failed or gave very bad yields, the direct oxidation of the alkaloid was attempted.

Oxidation with potassium permanganate did not give/

give any results. It was tried in acetone solution at room temperature and at 0°. When small amounts of the oxidising agent were used (less than 30 equiv.) the alkaloid was partially recovered, and with larger amounts of permanganate it was impossible to isolate anything. The oxidation in acid solution gave about the same results. Oxidation of an aqueous suspension of calycanthine at room temperature with potassium permanganate (30 equiv.) yielded only oxalic acid.

The alkaloid is very resistant to many of the oxidising agents. It can be boiled with calcium hypochlorite in the presence of cobalt nitrate, a combination that has been found to be a very powerful oxidising agent (Pictet and Patry, B., 1893, 26, 1962) being able to oxidise phenanthridine to phenanthridone.

The resistance of calycanthine against alkaline fusion is amazing. A sample that was refluxed for 12 hours with 30% potassium hydroxide in amyl alcoholic solution, was almost quantitatively recovered unchanged, although the glass of the flask was damaged to such an extent that the bottom was completely devitrified.

By fusion at higher temperature with sodium hydroxide alone, the alkaloid was partially decomposed, forming vapours with strong indole-like smell.

The/

The only substance that could be isolated was the unchanged starting material.

The oxidation with nitric acid yielded a crystalline compound $C_{17}H_7N_6O_{10}$ of very high m.p. (higher than 360°) and with acidic properties. The way in which this substance is formed shows clearly the resistance of calycanthine towards certain oxidising agents. In order to obtain this compound it was necessary to reflux the alkaloid with concentrated nitric acid for about 24 hours. If refluxed for a shorter time or diluted acid was used, amorphous nitro compounds, also with acid properties, were formed.

The crystalline substance obtained by nitric acid oxidation is probably a poly-nitro-carboxylic-acid. When heated in glycerol or quinoline solutions in the presence of copper bronze, carbon dioxide was given off, probably originated from a carboxylic group. It was tried to isolate the decarboxylated compound but no crystalline substance could be obtained. The nitric acid oxidation compound is slightly soluble in sodium carbonate, bicarbonate/

bicarbonate or hydroxide solutions. The red sodium salt which is formed is extremely little soluble in water, but can be recrystallised from this solvent in long hairy needles. It was impossible to do any further experiments with this nitric acid oxidation compound owing to its extreme insolubility in all solvents, except concentrated nitric acid and boiling nitrobenzene; the latter being used for purification.

In the oxidation with chromic acid (10 equiv.) in strong acetic acid solution, an amino acid was formed which could not be crystallised. Its methyl ester being easily formed could not be obtained in crystalline form. It was neither possible to crystallise any of the salts, nor derivatives of either the free amino acid or its methyl ester. Besides the amino acid another compound is formed. It has basic properties, its formula is $C_{16}H_{10}N_2$ and is identical with the zinc-dust distillation product. Attempts were made to oxidise calycanthine with a smaller amount of chromic acid. When to a solution of the alkaloid in dilute acetic acid two equivalents of chromic acid were added, a precipitate was formed. The so-formed substance that crystallises from boiling water is probably the chromate of the original base. Its aqueous solution gives reaction of free chromic acid/

acid.

The easiest way to explain the formation of the "zinc-dust distillation product" in the oxidation with chromic acid is to suppose that part of the molecule of calycanthine is oxidised. Dehydrogenation of the rest of the molecule takes place, giving a substance that is very easily decarboxylated yielding the compound mentioned above. This reaction can be compared with the formation of harmane (also oxygen-free) by oxidation with chromic acid of the condensation product of tryptophane with acetaldehyde (Kermack, Perkin and Robinson, loc. cit.).

Whilst the formation of the substance $C_{16}H_{10}N_2$ in the reactions carried out at high temperatures means little, the formation of the same substance by chromic acid oxidation of calycanthine makes it likely that in the molecule of this degradation product the original structure of the alkaloid is conserved unchanged. The formation of new rings and the migration of some atoms which sometimes happens in reactions taking place at high temperature, can be excluded.

That the $C_{16}H_{10}N_2$ compound has probably the same number of carbon atoms as the nitric acid oxidation compound suggests that in the molecule of the alkaloid must exist a nucleus or a grouping of carbon/

carbon atoms ready to form easily such a nucleus of sixteen carbon atoms which is rather resistant towards oxidation. Once dehydrogenated this skeleton of sixteen carbon atoms becomes very stable. The zinc-dust distillation product can be refluxed with concentrated nitric acid without being attacked at all.

The oxidation of calycanthine with hydrogen peroxide has been tried. In most of the alkaloids such an oxidation does not affect the molecule, only the amino oxide of the base being formed (e.g. M. and M. Polonowsky, C., 1926, II, 2309). In some cases the nor-alkaloid is formed (M. and M. Polonowsky, C., 1927, II, 2676), and in the case of ~~oxy~~cystine oxycystine is formed (Freund and Friedmann, Ber., 1901, 34, 605).

When calycanthine is suspended in hydrogen peroxide and heated on the water bath it dissolves. The solution does not precipitate with either acid or alkali and gives strong Mayer reaction. Probably an amino oxide has been formed. It can be obtained in amorphous form by evaporation, but it was never possible to regenerate the original alkaloid from it.

One of the methods that yielded more results in the chemistry of alkaloids is the Hofmann degradation/

degradation of the quaternary ammonium base obtained by exhaustive methylation.

In the case of calycanthine the preparation of the quaternary iodide is not very easy (Späth, loc. cit.) but by improving the method of preparation enough material was obtained without difficulty. When transformed to the ammonium base and distilled (or simply heated) in a high vacuum, trimethylamine evolution was observed. Although the distilled base could not be crystallised its methiodide was easily obtained in this form. It crystallises with a molecule of water. From the analysis of this methiodide can be concluded that one atom of nitrogen was lost.

The base obtained in the Hofmann degradation can be hydrogenated, because a double bond has been formed. The hydrogenated base could not be crystallised but its methiodide was easily obtained in the form of white needles.

These two facts, the formation of trimethylamine and a double bond, an atom of nitrogen being lost, show that in the molecule of calycanthine must be one group $NHCH_3$ in the immediate neighbourhood of a system of carbon atoms which can be involved in the formation of such a double bond.

The/

The existence of a primary amino group in the molecule of the alkaloid seems most unlikely. Evolution of nitrogen was not observed when calycanthine was treated with sodium nitrite. The analysis of the formed nitroso compound gives no evidence of this fact. Though easily obtained in a crystalline form with a sharp melting point, it is probably a mixture of the mono- and dinitroso-compound.

N-methyltryptamine obtained by Manske (loc. cit.) as benzoyl derivative has a $N\text{-}CH_3$ group in the side chain. This group is in such a position that it could be easily removed by Hofmann degradation. Is there in the calycanthine molecule a $CH_2\text{-}CH_2\text{-}N.CH_3$ open chain attached to the position 3 of an indole nucleus? No definite answer can be given to this question. Only the existence of such a chain would not agree with the resistance of calycanthine to some oxidising agents.

The methiodide of the base after the first step of the Hofmann degradation of calycanthine has taken place was transformed in the free base and again distilled in a high vacuum. No evolution of trimethylamine was now observed. If the ring with the/

the methylated N had opened in the first step of this degradation trimethylamine would now be formed. From the distillate which could only be obtained in amorphous form, a crystalline methiodide was prepared. This substance was proved to be identical with the methiodide of the base of the first Hofmann degradation step. The above results show that the N-atom which can be methylated and which is left after the first step of the degradation is still in its original position, i.e. either in the junction of two rings or the 1-position of an indole nucleus. In both cases the nitrogen could be methylated but the ring in which it is involved could not be opened in the first step of the degradation.

Calycanthidine.

The alkaloid isolated from the mother liquors of crystallisation of calycanthine crystallises in the form of well formed short rhombic prisms, with m.p. 126º. The name of calycanthidine is suggested for this new alkaloid.

Besides calycanthine the only alkaloid isolated from calycanthus species is the isocalycanthine of Gordin (J. Amer. Chem. Soc., 1909, 31, 1305). It is not very clear whether the two alkaloids are different; their formula is the same and the melting point only differs in 4°.

Calycanthidine, the new alkaloid isolated from the seeds of Calycanthus floridus L. is clearly different from calycanthine and isocalycanthine. It is present in a small amount; compared with 287 g. of calycanthine, only 27 g. of this new compound were isolated. The difference in the melting point of the two alkaloids is considerable. Calycanthine melts at 216° and the melting point of calycanthidine is 126°. Some of the solubilities also are different. Calycanthine is insoluble in petroleum ether and the new/

new alkaloid can be crystallised from this solvent - in fact it is one of the solvents from which it crystallises best. The crystalline form is also different; calycanthine crystallises in the form of tetrahedra and calycanthidine in the form of short prisms.

Although the new alkaloid is soluble in petroleum ether, the extraction of calycanthus seeds with this solvent did not remove any alkaloid. This makes it very likely that calycanthidine is in the seeds in the form of a salt.

The new alkaloid crystallises from wet solvents without water of crystallisation. The C, H and N analyses show that like calycanthine it is oxygen-free. The most likely formula for calycanthidine is either $C_{24}H_{30}N_2$, $C_{24}H_{32}N_4$ or $C_{25}H_{32}N_4$. N_4 ?

Molecular weight estimations carried out by the method of Rast were not very conclusive since the melting point of the solution of the alkaloid in camphor was not sharp. The active hydrogen estimation makes a formula half of the suggested very unlikely. Such a half formula is completely excluded owing to the fact that it has been possible to degradate the alkaloid to a compound with 16 C-atoms, identical with/

with that obtained from calycanthine. By zinc dust distillation the two alkaloids give the same compound $C_{16}H_{10}N_2$ and by nitric acid oxidation the same polynitro carboxylic acid. This suggests a similar skeleton in these two alkaloids.

The $N-CH_3$ estimation shows the presence of two such groups in the new alkaloid. That is one $N-CH_3$ more than in calycanthine. One of the extra carbon atoms can be accounted for in this way. The active hydrogen estimation shows the presence of one labile hydrogen atom. Practically the same amount of methane is formed at room temperature or when heated at 95°, unlike the case of calycanthine where the amount of methane formed at 95° is double that formed at 22°. Does that mean that the two hydrogen atoms which in calycanthine react with the Grignard reagent only at 95° are absent in calycanthidine?

All attempts to crystallise salts of the new alkaloid failed and only resins were obtained. Neither could crystalline acetyl or benzoyl products be obtained.

The methiodide is very easily formed. It is not necessary to heat, formation taking place at room/

room temperature in ether solution. This is different with calycanthine, which methiodide was only formed after prolonged heating of the alkaloid with methyl iodide at a rather high temperature. It may be that the difficulties in the methylation are somehow connected with the presence of the two not very reactive hydrogen atoms which react only with Grignard reagent at 95°.

The Hofmann degradation of the quaternary base obtained from the methiodide of calycanthidine also shows a difference with calycanthine. No trimethylamine is formed when the base is distilled. This shows that the $N-CH_3$ grouping of calycanthine does not exist in this alkaloid. It may be that it is involved in the formation of a new ring; perhaps one of the extra carbons is also involved in it.

Calycanthidine gives no Adamkiewitz-Hopkins reaction. As this reaction is characteristic for a free α-position of an indole nucleus (and positive in calycanthine) it seems possible that this particular position of the nucleus is substituted in the molecule of calycanthidine. Perhaps the closure of a ring corresponding to the ring C of harmane has taken place. No clear evidence supports this supposition and it is mainly based on negative results and colour reactions.

Experiments on the Alkaloids of Calycanthus floridus

Working up of the extract of Calycanthus seeds.

45 Kg. of Calycanthus floridus seeds are extracted with petroleum ether in order to remove fat and essential oils. After this extraction the seeds are percolated with alcohol. The alcoholic extract (reduced to a volume of about 3000 c.c.) is diluted with one litre of water, acidified with 100 c.c. hydrochloric acid and extracted with ether. Between both layers an emulsion is formed. The aqueous part is separated from the ethereal layer and the emulsion. The latter is destroyed by filtration and the so formed layers separated. The aqueous solutions are united and basified with a concentrated solution of sodium hydroxide. A white amorphous substance precipitates which becomes crystalline in a few minutes. By shaking the flask vigorously the large lumps are reduced to a crystalline powder. An oily layer appears covering the crystals floating on the surface of the alkaline aqueous solution. The whole content of the flask is filtered and the remaining solid (S) washed with water. The oily part passes easily through and remains/

remains on top of the filtrate. This oil is extracted with ether (O).

The solid part (S) is dissolved in hot acetone, boiled for a few minutes with 0.2 g. of charcoal and then filtered. To the hot solution water is added till a turbidity occurs and then allowed to cool at room temperature. Soon large crystals of calycanthine appear, which, after standing overnight, are filtered. By recrystallisation from acetone-water, the alkaloid is obtained in the form of well shaped tetrahedric crystals. The mother liquors of both crystallisations are mixed and the acetone removed by distillation on the water bath. Further amounts of crystalline material separate when the organic solvent is removed. From this second crop of alkaloid after several crystallisations from alcohol, two fractions are obtained: a more soluble part of m.p. 126° and a large quantity of calycanthine as the less soluble constituent.

The total amount of calycanthine was 287 g. = 0.64% of the seeds, and the yield of the alkaloid m.p. 126° was 27 g. = 0.06% of the seeds.

Analysis/

Experiments with Calycanthine

Analysis of Calycanthine.

The calycanthine so obtained crystallises in very well formed tetrahedra. It has a sharp m.p. of 216° which does not change by crystallisation from any water containing solvent. By prolonged heating at 120° in high vacuum over P_2O_5 or by crystallisation from very dry benzene the melting point can be raised to 242° (not sharp). No water of crystallisation can account for this fact. After drying at 120° in a high vacuum the loss of weight was extremely small. It was far below the calculated for one molecule of water of crystallisation. All the samples used for analysis were first crystallised several times from dry acetone or absolute alcohol and then dried to constant weight in a high vacuum under the conditions mentioned above.

Analysis

Found:	C, 75.04%	H, 7.47%	N, 15.9%
	75.07	7.65	15.75
	74.50	7.61	16.04
	73.83	7.68	16.05
	73.99	7.66	16.22
	73.71	7.53	16.15
	74.91	7.44	
	74.91	7.55	
	74.82	7.66	
Mean =	74.5	7.58	16.03
$C_{22}H_{26}N_4$	76.30	7.56	16.18
$C_{22}H_{28}N_4$ require	75.86	8.03	16.90

For the calculation of the further analysis the $C_{22}H_{26}N_4$ formula is used.

Active Hydrogen Estimation.

I.	5.635 mgm. subst. gave	0.79 c.c. CH_4,	reaction temp.	22°
		1.45 c.c. CH_4	do.	95°
II.	5.284 mgm. subst. gave	0.85 c.c. CH_4	do.	22°
		1.56 c.c. CH_4	do.	95°

Calc. for I (at 22°) 2.1 equiv. of active hydrogen
(at 95°) 3.9 do.
Calc. for II (at 22°) 2.0 do.
(at 95°) 3.7 do.

N-methyl estimations: Found 4.60 and 4.4% NCH_3

Calculated for 1 NCH_3 ... 4.4%

Distillation of calycanthine with zinc-dust.

1 g. finely powdered calycanthine is mixed with 30 g. of zinc dust and the mixture divided into ten portions. Each portion is put in the central part of a Pyrex glass tube (about 5 mm. wide and 15 cm. long) between two asbestos wool layers. The part of the tube occupied by the zinc dust is slowly heated with an open flame until the tube is red hot. The distillate formed in the two cold ends of the tube soon becomes partially crystalline. During the distillation gases with a strong indole-like smell are formed. They give a red colour to a split of pine wood previously impregnated with hydrochloric acid.

The/

The parts of the tubes containing the distillate are cut off and the distillation product extracted with a little acetone. The amorphous part is dissolved, the crystalline part remaining insoluble. The crystals are collected and washed with acetone and ether. The best way of purification for this substance is repeated sublimation in a high vacuum (bath temp. 180-200°) until no more residue is left. Three sublimations at least are necessary. By crystallisation from pyridine and recrystallisation from the same solvent the substance is obtained in long silky needles of m.p. 297°. 30-40 Mg. were obtained from each gram calycanthine.

This substance sublimes without the slightest decomposition below its m.p. 297°. It is extremely stable; a small portion was dissolved in fuming nitric acid and the acid evaporated on the water bath. After repeating this operation three times by sublimation of the residue, the unchanged starting material was recovered. The zinc-dust distillation product is soluble in mineral acids with yellow colour, but forms no salts. It can be extracted from an acetic acid solution by shaking/

shaking it repeatedly with ether.

Analysis.

Found:	C, 83.30%	H, 4.46%	N, 12.39%
	83.11	4.47	12.10
	83.04	4.63	12.24
Mean =	83.15	4.52	12.24

$C_{16}H_{10}N_2$
requires C,83.47 H, 4.38 N, 12.17

Active hydrogen estimation

4.760 mgm. subst. gave 0.42 c.c. CH_4, reaction temp. 23°
0.44 c.c. CH_4 do. 95°

Found 0.9 equivalent of active hydrogen.

The above carbon figures being slightly low may be due to the stability and volatility of the compound. A compound $C_{16}H_8N_2$ cannot be formulated.

The acetone used to remove zinc-dust distillation products from the tubes is evaporated to dryness. An oily residue with indole-like smell is left; this residue is dissolved in ether and extracted with hydrochloric acid. After separating both layers by decantation, the aqueous one was made alkaline. The precipitate was collected and sublimed in a high vacuum. A crystalline compound sublimes. After recrystallisation from dilute acetone it had m.p. 216°. The mixed melting point and the form of the crystals indicate that this basic substance is calycanthine which has survived the zinc-dust distillation. The ethereal layer

is/

is evaporated to dryness. The oily residue is distilled with steam and the distillate extracted with ether and dried with sodium sulphate. By evaporation on the water bath an oily residue with a strong indole-like smell was left. It distils at 120° (bath temperature) in a high vacuum. This substance could not be crystallised.

Dry distillation of calycanthine.

0.2 g. of the alkaloid placed on the bottom of a test tube is heated in a metal bath, the temperature being slowly raised to 360°. At 250° a liquid distillate begins to condense in the cold parts of the tube. When a temperature of 280-300° is reached the very characteristic tetrahedric crystals of calycanthine begin to appear in the distillate which consists now of two fractions. The less volatile portion is an oil mixed with the crystals of calycanthine. The more volatile fraction is a liquid. The whole distillate which has a strong indole-like smell, is extracted with ether. The larger part of it goes into solution but a small insoluble residue is left. The latter is collected by filtration and after being washed with fresh ether it is resublimed in a high vacuum at/

at 200° (bath temperature). It is obtained in the form of white needles of m.p. 297° and is identical with the zinc-dust distillation compound.

After filtration from the insoluble constituents the ether is evaporated to a small volume. By standing about 70 mg. calycanthine crystallise out.

Distillation of calycanthine with lead oxide.

0.1 g. of calycanthine is thoroughly mixed with 5 g. of red lead oxide. The mixture is distilled in the same way as the alkaloid was distilled with zinc dust. From the distillate could be isolated in small amounts (employing its insolubility in ether and purifying it by sublimation) the same substance of m.p. 297° obtained by zinc-dust and dry distillation. Besides this compound indole-like smelling amorphous substances were formed.

Distillation of calycanthine with copper oxide.

0.2 g. of calycanthine is mixed with enough copper oxide wire (as used for macro elementary analysis) to fill a 50 c.c. flask. The alkaloid is shaken with the copper oxide so that the small pieces of wire are covered with a very thin layer of alkaloid dust. The flask is heated in a metal

bath/

bath to a temperature of 320° for 3 hours. The sublimate formed in the cold parts of the flask is dissolved in a large amount of alcohol. This solution is evaporated to small bulk and diluted with ether; an amorphous substance precipitated. It is collected by filtration, washed with more ether and sublimed in vacuo. The sublimate consists of white needles, m.p. 300°. This substance is identical with the zinc-dust distillation compound.

From the filtered mixture of alcohol-ether by extraction with hydrochloric acid some unchanged calycanthine was recovered.

Baking of calycanthine with palladium black.

50 Mg. calycanthine and 100 mg. palladium black are thoroughly mixed and heated at 200-220° for 12 hours in a test tube. A sublimate is formed in the cold parts of the tube. This sublimate was resublimed in a high vacuum. The well formed crystals so obtained melted at 295° and are identical with the zinc-dust distillation product. The yield was poor, about 3 mg. of the resublimed material were obtained. No definite compound could be obtained by/

by extraction of the palladium residues with dilute hydrochloric acid or alcohol.

Selenium distillation of calycanthine.

Several attempts have been made to dehydrogenate calycanthine by means of selenium. Although different conditions (higher or lower temperature, longer or shorter heating periods) were used, no definite product could be obtained.

By heating 1 g. of calycanthine with 3 g. of selenium in a small retort at 300° a very little of a crystalline distillate was obtained. Resubliming this distillate and recrystallising it from pyridine it had a m.p. 297°. The substance so obtained is identical with the zinc-dust distillation product.

Oxidation of calycanthine with chromic acid.

10 g. of chromic acid dissolved in 15 c.c. of water and diluted with 50 c.c. of glacial acetic acid are added slowly to a solution of 10 g. calycanthine in 100 c.c. of glacial acetic acid. In the beginning a precipitate is formed, (probably the chromate of the alkaloid), but when more chromic acid is added the liquid becomes warm and homogeneous. The temperature was always kept below 25° by cooling.

Once/

Once the addition is finished the mixture is refluxed for five hours. The oxidation is now complete, no more chromic acid being detected by the hydrogen peroxide test.

When the reaction product is cold it is diluted with a little water and extracted ten times with ether. The united ether extracts are washed with sodium carbonate solution in order to remove the acetic acid that has been extracted and then dried with sodium sulphate.

The ether is evaporated on the water bath. Before becoming completely dry a crystalline solid begins to separate. When the residue is almost dry it is allowed to cool and the crystals filtered off. They are recrystallised twice from methyl alcohol, a solvent in which they are not very soluble. The purified substance is sublimed in a high vacuum (200° bath temperature). The sublimate crystallises from pyridine in the form of white silky needles, m.p. 295-298°, subliming without decomposition below the melting point. This substance has been found identical in all its properties with the zinc-dust distillation compound.

Analysis/

Analysis

Found:	C, 81.9%	H, 5.4%	N, 11.46%
	82.76	4.65	11.66
	83.16	4.44	11.76
$C_{16}H_{10}N_2$ requires	83.47	4.38	12.17

The chromic acid solution after being extracted with ether is evaporated to dryness in vacuo. The residue is dissolved in methyl alcohol, cooled at 0° and then saturated with hydrochloric acid gas. After standing for 48 hours at room temperature, the methyl alcohol is removed on the water bath. The oily residue is dissolved in water, alkalinised with sodium hydroxide and shaken out with ether.

From the ether solution by extraction with hydrochloric acid a substance with basic properties that gives Mayer's reaction, was extracted. It could not be crystallised either by direct crystallisation or after distillation in a high vacuum. It was also impossible to crystallise any of the derivatives or salts (picrate, methiodide, perchlorate, hydrochloride, etc.).

Oxidation /

Oxidation of calycanthine with concentrated nitric acid.

100 c.c. of concentrated nitric acid are added to 0.2 g. of calycanthine. During the addition of the first drops the mixture becomes very hot. When enough acid has been added to liquify the contents of the flask the rest can be added at once. A dark green solution is then formed. It is refluxed for 12 hours. In the beginning a large amount of nitrous vapours is formed but at the end hardly any more brown fumes are observed. The liquid is now pale yellow and crystals are formed in the bottom of the flask. After concentrating at normal pressure to a small volume, 25 c.c. of fuming nitric acid are added and again refluxed for 12 hours. By cooling well formed needles separate. They have a very high melting point, above 360° and begin to decompose at 330-340° without melting.

The substance is very insoluble. The only solvents from which it can be recrystallised are concentrated nitric acid and boiling nitrobenzene. The latter was used for recrystallisation.

Analysis

Found:	C, 44.02%	H, 1.52%	N,18.92%
	43.52	1.54	19.29
$C_{17}H_8O_{10}N_8$ requires	44.7	1.7	18.4

The/

The above mentioned compound is very sparingly soluble in cold sodium hydroxide or carbonate, but it is more soluble in the heat, giving a red solution. On cooling a hot saturated solution in sodium hydroxide, the formed sodium salt crystallises in long red hairy needles which can be recrystallised from water containing a little sodium hydroxide.

Oxidation of calycanthine with potassium permanganate

7 g. finely powdered calycanthine are suspended in 100 c.c. water. To this suspension a solution of 30 g. of potassium permanganate in 500 c.c. water are added slowly and shaking vigorously at the same time. Oxidation takes place rather quickly but without becoming too warm. After standing overnight the mass of manganese dioxide is filtered and thoroughly washed with hot water. The filtrate and the washing water are united, neutralised with hydrochloric acid and evaporated to dryness under reduced pressure.

The residue is extracted with acetone and after concentrating the acetone solution to a small bulk ether is added. A precipitate of inorganic material is formed which is filtered and washed with ether. The filtrate is evaporated to dryness and the/

the residue extracted with absolute ether. To this ethereal solution petroleum ether (40-50°) is added until a definite opalescence appears.

After standing overnight, crystals are formed which are collected by filtration, dried and sublimed in a high vacuum (100° bath temperature). The sublimate consists of a crystalline mass of m.p. 160°, which after standing for two days in the air, has a melting point of 105°. These crystals are soluble in water, alcohol, acetone and ether. When a few drops of calcium chloride solution are added to a solution of the above mentioned compound in dilute acetic acid, a white precipitate is formed.

This acid substance isolated from the potassium permanganate oxidation product is most likely oxalic acid.

Hofmann degradation of calycanthine.

Calycanthine methiodide. The methiodide was obtained by a method similar to the one used by Späth (loc. cit.). The slightly higher temperature used increased the yield to a practically quantitative one.

5 g. calycanthine and 10 c.c. methyliodide are/

are heated together at 130-140° for 15 hours in a sealed tube. After cooling the excess of methyl iodide is evaporated on the water bath and the dark mass of the methiodide dissolved in alcohol. It is important to remove as completely as possible the methyl iodide as the methiodide is completely insoluble in it. In experiments in which this precaution was not taken it was very difficult to extract the methiodide with alcohol.

The alcoholic solution is precipitated with an excess of ether. After standing overnight, the crude methiodide is collected by filtration. On recrystallisation from alcohol it is obtained in well formed needles.

Hofmann degradation of calycanthine methiodide.

1 g. of the methiodide is dissolved in about 300 c.c. of alcohol and mixed with the silver oxide which was previously prepared from 3 g. silver nitrate. The mixture is shaken until no more iodine ions can be detected with silver nitrate.

The silver iodide and the excess of oxide are removed by filtration. The filtrate is evaporated to dryness in vacuo. At the end of the evaporation an amorphous semi-solid substance begins to/

to separate which makes the solution froth intensively. In order to avoid this inconvenience a few drops of octyl alcohol were added. The residue is dissolved in the smallest possible amount of methyl alcohol, poured into a small round-bottom flask and the solvent eliminated on the water bath. Besides the smell of the alcohol a distinct basic smell is already noticed. The flask is now connected with the high vacuum pump and slowly heated in a metal bath. From 150° onwards a strong evolution of gases takes place. A piece of wet red litmus paper previously placed in the tube connected the flask with the pump, becomes blue.

After the temperature of 250° is reached the content of the flask is allowed to cool, extracted with alcohol and the solution concentrated to a small volume. Ether is added to this concentrated extract until no more precipitate is formed, and then filtered. To the filtrate an excess of methyl iodide is added and allowed to stand for 24 hours.

The formed amorphous precipitate is filtered. It is very hygroscopic but after crystallisation from/

from wet alcohol this property disappears and can be easily recrystallised from methyl alcohol. Precipitating the methyl alcoholic solution with ether the methiodide is obtained in a pure state, m.p. 180°. The yield was 0.2 g. of pure methiodide.

Analysis.

Found:	C, 52.62%	H, 6.24%	N, 8.29	I, 25.25%
$C_{23}H_{30}N_3I + H_2O$ requires	54.2	6.3	8.2	25.0
$C_{23}H_{29}N_3I + H_2O$ requires	53.3	6.5	8.5	25.6

As it was observed a certain amount of trimethylamine formed when the methyl alcoholic solution of the quaternary base was evaporated on the water bath, an attempt to remove trimethylamine under milder conditions was made.

1 g. calycanthine methiodide was refluxed for 10 hours with 20 c.c.30% sodium hydroxide. There is an evolution of trimethylamine. By extraction with ether only traces of an amorphous basic substance were isolated.

The degradation can also be carried out in the way that the formed base is distilled. It distils at 260-270° (bath temperature) under a pressure/

pressure of 2 mm. but this method gives a poor yield. Besides the oily base a white crystalline product distils. The latter is sparingly soluble in alcohol and ether. After sublimation and re-crystallisation from pyridine it has a melting point of 295°. This compound is probably the same as that obtained by zinc-dust distillation.

Hydrogenation of the Hofmann degradation product of calycanthine.

1.4 g. of the oily base obtained by distillation of the free base of calycanthine methiodide are dissolved in about 5 c.c. of glacial acetic acid and shaken in a hydrogen atmosphere with 0.1 g. platinum oxide (after Adams). Absorption of 120 c.c. of hydrogen was observed. Calculated for 0.1 g. platinum 20 c.c. H_2; calculated for one double bond 80 c.c.

The acetic acid solution is diluted with water and filtered from the platinum. By alkalinisation an amorphous precipitate is formed. Several attempts were made to crystallise it but without any success.

1 g. of this base is dissolved in ether and an excess of methyl iodide added. After standing for/

for 6 hours the precipitate is collected and crystallised once from alcohol and once from water, m.p. 323°.

Experiments with Calycanthidine.

The alkaloid isolated from the mother liquor of the crystallisation of calycanthine is re-crystallised twice from alcohol and once from petroleum ether (80-100°). Its melting point does not change, remaining at 126°. The alkaloid crystallises in short rhombic prisms.

When heated in a high vacuum it distils but does not sublime unless this distillation is carried out extremely slowly in which case the formation of crystals is observed. The taste of the alkaloid is very bitter. It is practically insoluble in water and is soluble in all the tried solvents. It becomes yellowish in the course of time by standing.

Analysis.

Found:	C, 76.33%	H, 7.83%	N, 14.68%
	76.75	8.19	14.69
	76.82	8.20	
	76.15	8.02	
	Mean =	8.06	
Calc. for $C_{24}H_{30}N_4$	77.0	8.02	15.0
$C_{24}H_{32}N_4$	76.6	8.5	14.9
$C_{25}H_{32}N_4$	77.3	8.25	14.43

Active hydrogen estimation.

I. 8.855 mgm. subst. gave 0.50 c.c. CH_4, reaction temp. 21°
0.54 c.c. CH_4 do. 95°

II. 7.410 mgm. subst. gave 0.43 c.c. CH_4 do. 21°
0.45 c.c. CH_4 do. 95°

Calculated for I: 0.9 equivalents of active hydrogen.

Calculated for II: 1.0 equivalents of active hydrogen.

N-CH_3 determination

Found: 7.43 and 7.24% NCH_3

Calc. for 2 N-CH_3 7.9%

Optical rotation

Found: $[\alpha]_D$ = -144° (alcoholic solution c = 0.079 l=2)

The acid solution of the alkaloid gives strong Ehrlich reaction but in contrast to calycanthine no glyoxilic reaction. No crystalline salts could be obtained with inorganic acids, resins being always formed.

The picrate is easily obtained by dissolving 0.5 g. of the alkaloid in 10 c.c. alcohol and adding to this solution 50 c.c. of a concentrated solution of picric acid in alcohol. The precipitate is then filtered and recrystallised from acetone, a solvent in which it is rather soluble. The picrate crystallises/

crystallises in the form of yellow needles, m.p. 192° which do not change after further crystallisation.

Analysis

Found:	C, 51.23%	H, 4.55%	N, 16.40
Calc. for			
$2C_6H_3O_7N_3 + C_{24}H_{36}N_4$	51.5	5.0	16.7
$C_{24}H_{36}N_4 + 2C_6H_3O_7N_3$	51.9	4.33	16.8
$C_{25}H_{32}N_4 + 2C_6H_3O_7N_3$	52.5	4.4	16.5

Methiodide.

2 g. alkaloid are dissolved in 200 c.c. ether and an excess of methyl iodide added. In about ten minutes an opalescence appears. After 48 hours the formation of a white crystalline precipitate is finished. After crystallisation from methyl alcohol is has m.p. 210°.

Analysis

Found:	C, 46.54%	H, 5.91%	N, 8.46%	I, 39.39%
Calc. for				
$C_{24}H_{32}N_4 + 2ICH_3$	47.0	5.7	8.4	38.4
$C_{25}H_{32}N_4 + 2ICH_3$	48.0	5.6	8.3	38.1

Nitroso/

Nitroso derivative.

0.5 g. alkaloid is dissolved in about 20 c.c. of dilute acetic acid, and an excess of a saturated aqueous solution of sodium nitrite is added. The amorphous precipitate which is formed is filtered. It is dissolved in pyridine and heated to boiling. Water is then added until a slight opalescence appears and allowed to cool. The nitroso derivative separates in the form of an oil which becomes crystalline after standing. Recrystallised from pyridine water without using an excess of water it can be obtained in the form of yellow needles, m.p. 75-78°, probably a mixture.

ON THE CONSTITUTION OF ASPIDOSPERMINE

The alkaloid aspidospermine has been isolated from the barks of the Apocyanaceae Aspidosperma quebracho, a South American plant (Fraude, Ber., 1878, 11, 2189). It is not the only alkaloid present in this plant (Hesse, A., 1888, 211, 249). Two other well defined alkaloids have been isolated from it. They are quebrachamine and quebrachine. The latter has been proved to be identical with yohimbine. (Fourneau and Page, Bull. Sci. Pharmacol. 1914, 21, 7; see also footnote, Ewins, J. 1914, 105, 2729).

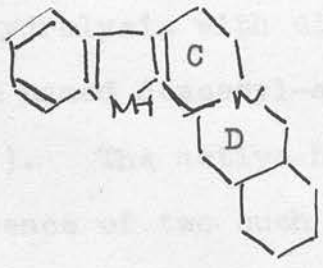
Quebrachamine is an oxygen-free compound and aspidospermine has two atoms of oxygen in its molecule. The formula of aspidospermine was established by Fraude (loc. cit.) and confirmed by Hesse (loc. cit.). It is $C_{22}H_{30}O_2N_2$.

The estimation of the methoxy groups shows one of these groups in the molecule and the determination of NCH_3 indicates the absence of such a group. By hydrolysis with dilute hydrochloric acid aspidospermine loses an acetyl group forming a new/

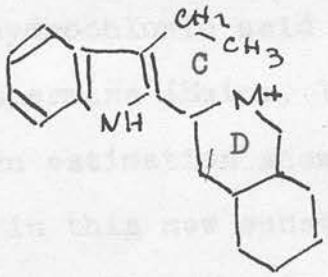
new base, much more basic from which by acetylation aspidospermine is regenerated (Ewins, loc. cit.)

The formulas of aspidospermine $C_{19}H_{26}N_2$, $CH_2CO.CH_2O$ and yohimbine $C_{19}H_{24}N_2$, $C_2H_2O_2.O$ are closely related. The two can be derived from the same skeleton $C_{19}H_{24}N_2$ which in the case of yohimbine has a carbomethoxy ($C_2H_2O_2$) and a hydroxyl (O). The same skeleton occurs in aspidospermine but is hydrogenated ($C_{19}H_{24+2}N_2$) which is the bearer of a methoxyl (CH_2O) and of a N-acetyl (CH_2CO).

The occurrence of both alkaloids together in the same plant also suggests a relation between them. In the skeleton of yohimbine is no double bond which could be hydrogenated without losing the characteristic indole group. The two extra hydrogen atoms of aspidospermine can be accounted for the opening of one of the rings of the yohimbine molecule.



Yohimbine skeleton



Aspidospermine skeleton

Aspidospermine does not form a methiodide nor give a nitroso derivative. It forms no salts and the basic properties of the alkaloid are very weak. When a pyridine solution is allowed to react at room temperature with methyl-magnesium-iodide (active hydrogen estimation after Zeriwitinoff), no methane is formed, but if the mixture is heated at 95° the amount of methane now formed fits exactly for the calculated for one active hydrogen. The presence of an active hydrogen in the molecule of the alkaloid supports the supposition that one of the rings of the yohimbine skeleton is open in this alkaloid, and that this ring is either C or D. An extra secondary nitrogen atom is necessary in order to place the N-acetyl group of aspidospermine besides a free NH group in the molecule of the alkaloid.

The substance obtained from aspidospermine by hydrolysis with dilute hydrochloric acid has been named deacetyl-aspidospermine (Ewins, loc. cit.). The active hydrogen estimation shows the presence of two such atoms in this new substance. It forms easily a methiodide. When the free quaternary ammonium base obtained from it is distilled an oily base is obtained. It could not be/

be crystallised but its methiodide has been proved to be identical with the methiodide of deacetyl-aspidospermine. No ring has been opened in this first step of the Hofmann degradation. Deacetyl-aspidospermine reacts with nitrous acid, yielding different compounds, if the reaction is done in acetic acid solution or in the presence of hydrochloric acid. In the first case the reaction compound has m.p. 160° (it had been prepared before by Ewins, loc. cit.) and a formula $C_{20}H_{28}O_4N_4$ which means that NO_2 has entered the molecule. The reaction product of deacetyl-aspidospermine with nitrous acid in the presence of hydrochloric acid has m.p. 226°. Its formula is $C_{20}H_{28}O_5N_4$ or $C_{20}H_{30}O_5N_4$. In the base N_2O_2 or $N_2O_2H_2$ has entered. The difference between the two nitroso compounds could be H_2O, the elements of water, but it is not a molecule of water of crystallisation. The two nitroso compounds when refluxed with hydriodic acid (1.7) yield the same phenolic compound aspidospermine, also directly obtained from aspidospermine by a similar treatment (Ewins, loc. cit). This means that no changes have taken place in the original structure of the alkaloid molecule by the action of nitrous acid.

Deacetyl/

Deacetyl-aspidospermine can be regenerated from the nitroso compound (m.p. 160°) by boiling with dilute hydrochloric acid.

When deacetyl-aspidospermine is refluxed with formic acid a new base is formed. This new compound is the formylation product of deacetyl-aspidospermine. It gives no bright red colour by treatment with oxidising agents as does deacetyl-aspidospermine. By hydrolysis with dilute hydrochloric acid deacetyl-aspidospermine is regenerated from this compound. Formyl-aspidospermine forms no methiodide, reacting with methyliodide as deacetyl-aspidospermine. It reacts with nitrous acid giving different compounds when the reaction is carried out in the presence of acetic or hydrochloric acid. The melting point of the nitroso compound in the first case is 185°, and in the second 270° and has a formula $C_{21}H_{30}O_5N_3$, which means an increase of O_2N. Both nitroso compounds are very unstable against alkali. When treated with cold dilute sodium hydroxide the one of m.p. 270° gives deacetyl-aspidospermine (the formyl group being also hydrolysed), and the one of m.p. yields formyl-aspidospermine.

The active hydrogen of aspidospermine seems very unreactive. It forms no methiodide or nitroso compound/

compound and reacts with methyl magnesium iodide only in the heat. In formyl-aspidospermine in which the acetyl group is replaced by a formyl, the active hydrogen becomes a little more reactive. It forms a labile nitroso compound but no methiodide. Deacetyl-aspidospermine shows all the reactions of a secondary base, reacts with nitrous acid, forms a methiodide and reacts with methyl magnesium iodide at room temperature. When treated with some oxidising agents (for instance nitric acid) it gives a deep red colour, a property which aspidospermine and formyl-aspidospermine have not.

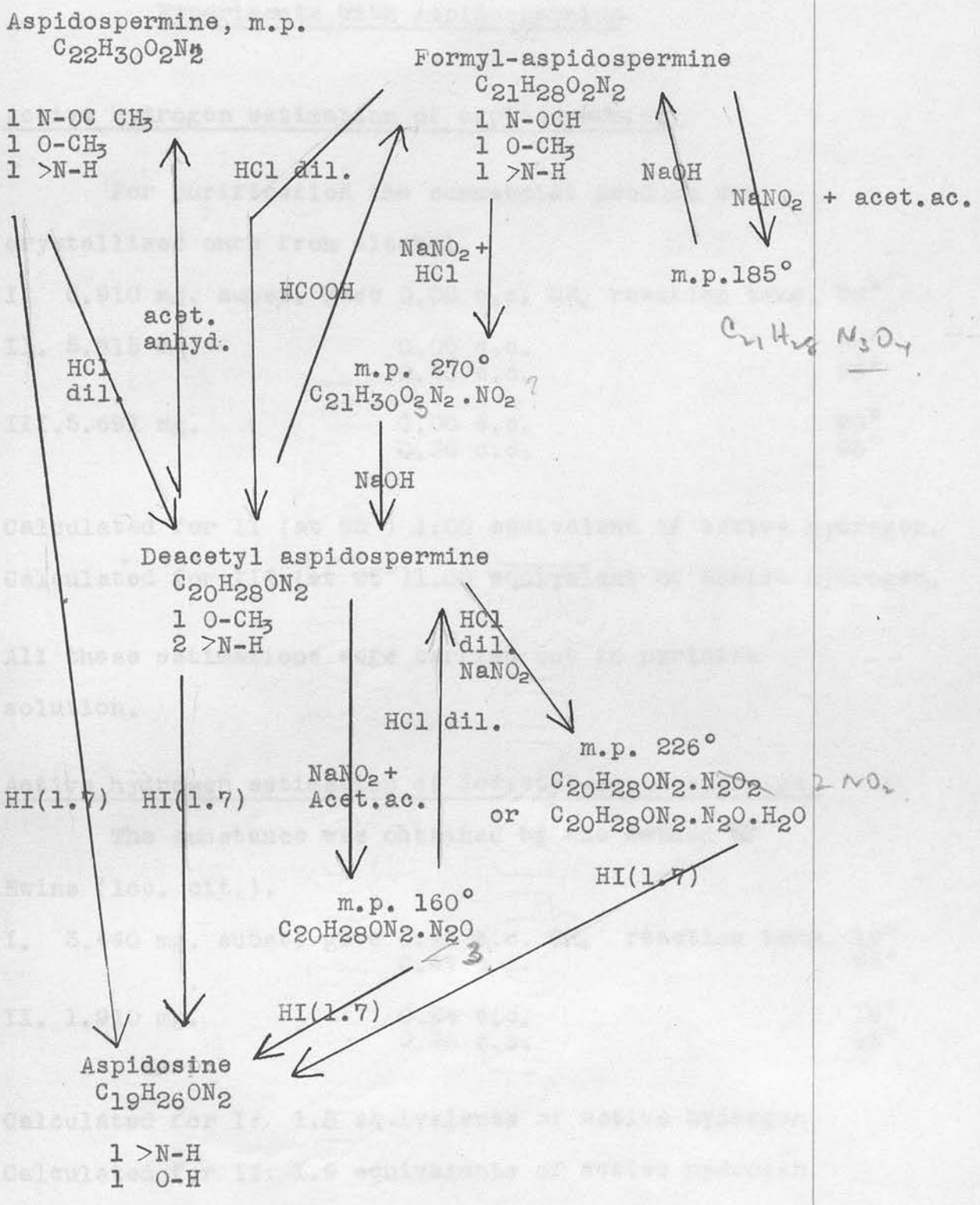
The methoxy group of aspidospermine can be removed by hydrolysis with hydriodic acid (1.7), the acetyl group being also hydrolysed. The resulting compound has phenolic properties. It is soluble in sodium or potassium hydroxide and to some extent in ammonia or sodium carbonate.

From its formula $C_{19}H_{28}ON_2$ it seems that only the O-methyl and the N-acetyl have been eliminated, but the active hydrogen estimation shows that further changes have taken place in the molecule. Aspidospermine should have 3 active hydrogens, one already present in aspidospermine and the other two, the points/

points in which the N-acetyl and O-methyl were attached. Estimations carried out at room temperature and at 95° gave only two labile hydrogen atoms. The analysis figures are too low even for two atoms, but this may be due to partial neutralisation of the basic N with the phenolic hydroxide.

It was tried to transform aspidosine in deacetyl-aspidospermine by methylation with diazomethane. Only an uncrystallisable oil was obtained as reaction product. The same result gave the methylation with dimethylsulphate. Deacetyl-aspidospermine does not react with diazomethane which makes it unlikely that the methylation compound of aspidosine is a N-methyl derivative of deacetyl-aspidospermine.

The key to the structure of yohimbine was given by the study of its selenium dehydrogenation compounds. The selenium dehydrogenation of aspidospermine and aspidosine was tried but no crystalline substance could be obtained. The zinc dust distillation of aspidospermine also failed. The only compound which could be isolated from the distillate was a small amount of the unchanged starting material.


Aspidospermine, m.p.
C22H30O2N2
1 N-OC CH3
1 O-CH3
1 >N-H
HCl dil.
Formyl-aspidospermine
C21H28O2N2
1 N-OCH
1 O-CH3
1 >N-H
NaOH
NaNO2 + acet.ac.
m.p.185°
NaNO2 + HCl
HCOOH
acet. anhyd.
HCl dil.
m.p. 270°
C21H30O3N2.NO2
NaOH
Deacetyl aspidospermine
C20H28ON2
1 O-CH3
2 >N-H
HCl dil. NaNO2
HCl dil.
m.p. 226°
C20H28ON2.N2O2
or C20H28ON2.N2O.H2O
NaNO2 + Acet.ac.
HI(1.7)
HI(1.7)
HI(1.7)
m.p. 160°
C20H28ON2.N2O
HI(1.7)
Aspidosine
C19H26ON2
1 >N-H
1 O-H

Experiments with Aspidospermine

Active hydrogen estimation of aspidospermine

For purification the commercial product was crystallised once from alcohol.

I.	6.910 mg. subst. gave	0.02 c.c. CH_4	reaction temp. 20°
II.	5.815 mg.	0.00 c.c.	20°
		0.40 c.c.	95°
III.	5.691 mg.	0.00 c.c.	20°
		0.36 c.c.	95°

Calculated for II (at 95°) 1.00 equivalent of active hydrogen.

Calculated for III (at 95°)1.00 equivalent of active hydrogen.

All these estimations were carried out in pyridine solution.

Active hydrogen estimation of deacetyl-aspidospermine.

The substance was obtained by the method of Ewins (loc. cit.).

I.	3.440 mg. subst. gave	0.46 c.c. CH_4	reaction temp. 19°
		0.47 c.c.	95°
II.	1.910 mg.	0.24 c.c.	19°
		0.26 c.c.	95°

Calculated for I: 1.8 equivalents of active hydrogen

Calculated for II: 1.9 equivalents of active hydrogen.

Active hydrogen estimations of aspidosine.

The substance was prepared after Ewins (loc. cit.).

I.	7.027 mg. subst. gave	0.88 c.c. CH_4	(room temp.)
II.	8.048 mg.	0.99 c.c. CH_4	do.
III.	6.660 mg.	0.81 c.c.	reaction temp. 18°
		0.95 c.c.	do. 95°

Calculated for I: 1.57 equivalents of active hydrogen.

II: 1.60 do. (at 18°)

III: 1.86 do. (at 95°)

All the estimations were done in pyridine solution.

Action of nitrous acid on deacetyl-aspidospermine.

To 0.2 g. deacetyl-aspidospermine (prepared after Ewins, loc. cit.) dissolved in 5 c.c. of 2N hydrochloric acid, is added a saturated aqueous solution of sodium nitrite until no more precipitation takes place. It is allowed to stand for one hour. The precipitate is filtered and crystallised from alcohol, m.p. 224-226° which remains constant after three crystallisations from this solvent.

Analysis/

Analysis.

Found:	C, 59.30%	H, 7.04%	N, 13.25%
Calc. for $C_{20}H_{28}O_5N_4$	59.4	6.9	13.86
$C_{20}H_{30}O_5N_4$	59.1	7.29	13.79

0.2 g. of deacetyl-aspidospermine is dissolved in 2 c.c. 10% acetic acid and a saturated solution of sodium nitrite added until no more precipitate appears. It was then filtered and crystallised from alcohol, m.p. 160° (Ewins 156-158°).

Analysis

Found:	C, 60.33%	H, 6.58%	N, 14.32%
Calc. for $C_{20}H_{28}O_4N_4$	61.8	7.23	14.5

The above results are in accordance with the statement of Ewins that this nitroso compound is not easily analysed.

Action of dilute hydrochloric acid on the nitroso-deacetyl-aspidospermine of m.p. 160°.

0.1 g. of the nitroso compound, m.p. 160° is refluxed for 3 hours with 0.5 c.c. concentrated hydrochloric acid diluted with 3 c.c. alcohol. After cooling water is added and the alcohol removed by distillation. The remaining aqueous solution is allowed/

allowed to cool and made alkaline with sodium hydroxide. A precipitate appears which is shaken out with ether. The ether solution is dried with sodium sulphate and evaporated on the water bath. The residue is distilled in a high vacuum (120° bath temperature) and the distillate crystallised from acetone-water, m.p. 100°, mixed m.p. with deacetyl-aspidospermine (107°) is 108°.

The nitroso-deacetyl-aspidospermine of m.p. 226° boiled with hydrochloric acid under the same conditions yielded a resin which could not be crystallised nor distilled.

Action of hydriodic acid (d = 1.7) on the nitroso-deacetyl-aspidospermine of m.p. 160°.

0.1 g. of the nitroso-deacetyl-aspidospermine is refluxed for one and a half hours with 3 c.c. hydriodic acid. The latter is removed by distillation in vacuo, the residue suspended in a little water and decolorised with SO_2. By alkalinisation with ammonia a precipitate is formed. It is filtered and crystallised from acetone-water, m.p. 240° (no depression when mixed with aspidosine).

Action/

Action of hydriodic acid (d = 1.7) on nitroso-deacetyl-aspidospermine of m.p. 226°.

0.05 g. of the nitroso compound is refluxed for 5 hours with hydriodic acid (d = 1.7). The hydriodic acid is evaporated in vacuo, the residue suspended in a few drops of water and decolorised with SO_2. The clear solution is made alkaline with ammonia and extracted with ether. The ether solution is dried with sodium sulphate and evaporated on the water bath. The residue is distilled in a high vacuum (200° bath temperature). The crystalline distillate melts at 235°, giving no depression when mixed with aspidosine.

Formylation of deacetyl-aspidospermine.

0.1 g. deacetyl-aspidospermine is refluxed with 3 c.c. formic acid for 3 hours, and then diluted with water and made alkaline with sodium hydroxide. The white precipitate is collected and crystallised from acetone water. White needles, m.p. 153°.

Analysis

Found:	C, 74.87%	H, 8.01%	N,8.11%
Calc. for $C_{21}H_{28}O_2N_2$	74.1	8.2	8.2

The/

The substance does not give the typical coloration of deacetyl-aspidospermine when treated with dilute nitric acid, neither does it form a methiodide when its ethereal solution is allowed to stand with methyl iodide.

A small amount of this formyl-aspidospermine is refluxed with dilute hydrochloric acid for three hours, and the solution made alkaline with sodium hydroxide. The precipitate is then collected and crystallised from acetone, m.p. 109°; no depression was observed when mixed with deacetyl-aspidospermine.

Another small amount of formyl-aspidospermine is refluxed for one hour with hydriodic acid (d = 1.7). After diluting with water by careful neutralisation with ammonia a precipitate is formed. After crystallising it from acetone it had m.p. 236° and no depression with aspidosine.

Action of nitrous acid on formyl-aspidospermine in the presence of hydrochloric acid.

A small amount of formyl-aspidospermine is dissolved in a little hydrochloric acid and on adding a saturated aqueous solution of sodium nitrite a precipitate is formed. After standing for two hours the precipitate is collected by filtration and crystallised from alcohol. Yellow needles, m.p. 270°.

Analysis/

Analysis

Found:	C, 63.41%	H, 7.42%	N,10.30%
Calc. for $C_{21}H_{30}O_5N_3$	62.4	7.4	10.4

This nitroso compound is soluble in water so when the reaction is carried out with a dilute solution of the formyl compound the precipitation of the nitroso compound is very slow and only partial. When to an alcoholic solution of the nitroso compound water is added no precipitation takes place, but if 2N sodium hydroxide solution is added, a precipitate is formed. This precipitate is soluble in dilute hydrochloric acid and appears again when the solution is made alkaline with sodium hydroxide. It can be shaken out with ether from the alkaline solution and from the ether solution extracted with hydrochloric acid. When the solution in hydrochloric acid of the basic substance so obtained is make alkaline with sodium hydroxide, a cloudy yellowish precipitate appears which is filtered and dried. It distils in the form of a thick oil in the high cacuum (100-110° bath temp.). The distillate is crystallised from acetone and recrystallised from acetone water. White needles of m.p. 107° are obtained/

obtained which give no depression when mixed with deacetyl-aspidospermine.

Action of nitrous acid on formyl-aspidospermine in the presence of acetic acid.

To a small amount of formyl-aspidospermine dissolved in dilute acetic acid a saturated solution of sodium nitrite is added. After a few minutes a precipitate appears which becomes crystalline in an hour. Recrystallised from acetone it has m.p. 185°.

Analysis.

Found: N, 10.89%

Calc. for $C_{21}H_{28}O_4N_3$ 10.9%

The substance is soluble in acetone and alcohol. When water is added to one of these solutions no precipitation takes place, but if 2N sodium hydroxide is added, a crystalline precipitate is formed. After recrystallisation from acetone m.p. 148° it shows no depression when mixed with formyl-aspidospermine.